Evaluation of sorghum in gluten-free soy sauce

by

Cole Pearson

B.S., Kansas State University, 2012

A REPORT

submitted in partial fulfillment of the requirements for the degree

MASTER OF SCIENCE

Food Science
College of Agriculture

KANSAS STATE UNIVERSITY
Manhattan, Kansas

2017

Approved by:

Major Professor
Dr. Fadi Aramouni

Copyright

Abstract

Gluten-free products are becoming more prevalent in the market today, however there are a few types of products that have “hidden” gluten and people will not realize until after consumption. Products like soy sauce and beer are sources of gluten that people don’t know about. Soy sauce contains wheat as a main ingredient so replacing it with a gluten-free flour such as sorghum may produce a product similar to wheat-based soy sauce. Sorghum was used in this experiment since it is a grain grown in the mid-western region of the United States and a growing food ingredient in the global market. Sorghum can come in many different varieties and colors so we used different varieties in this study. Four treatments were done using three different sorghum flours (black, white, and waxy sorghum flour) and a wheat flour for a control. Cooked soybeans were mashed in a kitchenaid mixer and the treatment flour was added to make a dough. That dough was formed into a log and cut into slices. The slices were then staked with wet paper towels to mold. After 13 days of molding, the slices that were made were dried, placed in a salt solution, and fermented for 100 days. The solution was mixed with a spatula for 30 seconds to homogenize the mixture every 2-3 days and samples were taken every 10 days to test for pH, salinity, and color. Once the 100 days were complete and the pH of each treatment did not drop for consecutive testing periods, the liquid was removed from the solids and pasteurized. The pH curve did show that a fermentation process did occur, however there was no control over what microorganism could grow. Consumer testing was not performed since all test sauces were deemed unacceptable at the initial screening.

Table of Contents

List of Figures

List of Tables

Chapter 1 - Literature Review

1.1 Celiac Disease

The prevalence of gluten-free foods has come to a high point due to the number of people who have celiac disease, non-celiac gluten sensitivity NCGS, and wheat allergies. Celiac disease is an autoimmune disease that affects the body's ability to absorb or digest gluten that is found in wheat and certain grains that contain the proteins gliadin and glutenin (Green et al. 2015). The issue happens mainly in the intestines where the proteins will interact with the villa on the wall of the intestines and can cause inflammation and breakdown of the villa. Celiac disease, which can affect up to 1% of the U.S. population or about 30 million people out of 300 million in the United States, can cause sufferers to have symptoms of bowel irritation, diarrhea, rash, abdominal discomfort, and fatigue (Green et al. 2015). While genetics do have an impact on whether a person will have celiac disease, factors such as the environment and the season when a person was born can contribute to the symptoms of the disease. The percentage of certain population with celiac disease differs from country to country (Green et al. 2015). Countries in Europe such as Germany have a 0.3% rate of celiac while England has a 1.2% rate and Finland has a 2.4% rate. Areas that had more traditional gluten-free food before the world trade market and have now incorporated wheat based foods into the diets could have some individuals develop celiac problems (Green et al. 2015).

NCGS can sometimes be confused with celiac disease as the main symptoms that patients have can overlap with symptoms of celiac disease such as diarrhea, fatigue, and abdominal pain. Unlike celiac disease, NCGS does not come from history and genetics. Both celiac disease and non-celiac gluten sensitivity can be set on by the ingestion of gluten within minutes or hours. There has been some disagreement as to what extent the NCGS is as a clinical diagnosis (Green et al. 2015).

The final group of people who follow gluten-free diets due to health reasons are people with wheat allergies. This allergy is not based on the gluten protein but all other parts of the wheat protein and other parts such as certain enzymes and starches. The wheat allergy is included on the FDA's list of top 8 allergens that need to be listed on ingredient statements of all food items sold to the public. All of the above categories; celiac disease, non-celiac gluten sensitivity, and wheat allergy have reasons to have a gluten-free diet in their daily life.

Additionally, some people are pushing gluten-free diet as a healthier substitute than the normal conventional diet that contains gluten. The problem with substituting gluten out of certain foods such as bread or other baked products is that gums and other fats and sugars are used to try to replicate the effects of gluten in the products. This can cause gluten-free products to have a higher calorie intake than the conventional product (Reilly 2016). Another issue is that gluten-free flours especially are not enriched like most wheat flours. This is to replace nutrients that the milling process can remove. Most whole wheat flours do have certain vitamins and minerals, however all wheat flour that is not whole grain are missing these nutrients. They are added up to the amount whole grain flour would have. Enriched flour is wheat flour that has additional nutrients added at a higher level than what would originally be in the wheat kernel as stated in CFR title 21 part 104 subsection B (CFR 2016). This can cause people to have deficiencies in certain vitamins and minerals such as folate and iron (Reilly 2016).

While most of the deficiencies can be taken care of by taking a multi vitamin, using certain gluten-free flours can cause a greater risk of exposure to certain toxins such as arsenic or mercury (Reilly 2016). Rice is a very good source to find arsenic as it is frequently used in gluten-free foods. Rice easily uptakes arsenic from the environment and can be found in whole rice and the flour as well (Reilly 2016).

1.2 Gluten Substitutes

Removing wheat flour or wheat ingredients can be difficult in a recipe because, gluten has many functions in a product such as dough structure. To overcome this in a formulation, many companies will replace wheat flour with other gluten-free flours such as rice, potato, sorghum, and other minor grains such as teff, buckwheat, and millet. Other starches that can also be used instead of wheat starch are: tapioca, corn, and rice. These flours can easily replace wheat in a formula. However, the gluten protein is essential for dough structure, elasticity, and water retention (Lafiandra 2004). The proteins found in the other flours do not function in the same way, hence the need for functional additives.

Gums have a wide variety of uses in the food industry, some can be used in food such as ice creams while, others are used in baked or heated products such as bread or confections. The type of gum is important as to how it will function in a specific food item. Gums can provide structure and elasticity, however, gums can cause the product to dry out faster. Gums can be made from plants or microbial fermentation. These include guar gum, xanthan gum, locust bean, cellulose gum, and carrageenan (Gums and Stabilizers in the Food Industry Conference 2012). Each one has a particular food that they are best suited. Gums also do not add much in terms of calories in the recipe.

Another ingredient that is used in gluten-free foods is fats. Fats such as liquid oils such as sunflower and peanut oil and solid fats such as hydrogenated oil and lard are a good way to bind ingredients together and retain moisture in the product. However, fats do not do a good job in terms of structure and elasticity (Sato and Ueno 2014). Fats added to recipes provide additional calories which may be a concern for people that consume the products. Obesity, overweight, and new- onset insulin resistant have been identified with patients that have followed a gluten-free diet (Reilly 2012). This can be a concern for people that are looking at gluten-free as a healthier alternative to conventional products.

For the most part, the gluten-free products are mainly baked breads or bakery items that use the above additives to replace the gluten that is found in wheat flour. Early gluten-free products were hard on consumers as there was not many varieties, as well as, a short shelf-life that would leave the product stale and hard to consume. There are large companies that specialize in gluten-free products. One such company is UDI's, Boulder Colorado. This company has a large portfolio of products that it provides to consumers. Products include: bread, bagels, baguettes, muffins, several types of cookies, tortillas, pizza crusts, and buns. Gluten-free food companies will conduct research and development to find the right blend of gluten-free flours and additives to make a product that is appealing to consumers and has a longer shelf life and can be comparable to conventional gluten containing products. These products will have mostly gums, sugars, and different gluten free flours and starches that make them different. Gluten-free products are becoming more like the gluten counterparts and will continue to improve with research into different ingredients that will improve functionability in the same way that gluten does.

1.3 Other Gluten Containing Products

However, there are several products that do not seem to fit in the category of gluten containing products. These products make it hard for a consumer to tell if they contain gluten without reading the ingredients. Many of these products are liquids or semi-solids that use wheat flour or wheat starch as a thickening agent in soups, marinades, and dressings. The use of wheat flour or wheat starch can be a small percentage in the formulation and people who are less sensitive to gluten may not have any issues or symptoms to show that the food item has gluten in it.

Other products that can contain gluten are liquids that use wheat or gluten-containing grains as a main ingredient to make the product. Products in this category are many types of beer and traditional products such as soy sauce. Beer is a hidden source of gluten that most people do not realize till after they consume the product and show symptoms. The production of most types of beer use barley or wheat in raw or malted form that are soaked in hot water to extract sugars and other parts of the grain that are needed for flavor and beer fermentation (Tanner et al. 2013). These steps are called mashing of the grain and lautering of the grain. In these steps, water and temperature are used to extract the sugars, however, if extraction takes longer than normal then proteins can be extracted by the water. Some styles of beer are filtered after fermentation to remove proteins that can cause beer haze, however not all proteins are removed. Styles of beer that are made with fruit or grains such as sorghum or rice can make a gluten-free beer for people with celiac disease or gluten sensitivity.

1.4 Soy Sauce

The other product of concern is soy sauce. Main ingredients in traditionally made soy sauce are soybeans, wheat or wheat flour, salt, and water. The simple formulation combine soybeans and wheat to become the base of the soy sauce that will be fermented in a salt water solution. As the base sits in the salt solution, starches and proteins can leach out and be in the final sauce. The base is first subjected to molds over a two week period. These molds will breakdown the starches into fermentable sugars and breakdown proteins and cellulose for yeast to use during fermentation (O'Toole 1997).

The history of soy sauce is found in the Far East. Most scholars will say that it originated in China with a product similar to soy sauce. This product, called chiang, was moldy grain that had yellow *Aspergilli* molding on its surface. It was mainly millet along with animal or fish flesh, and salt that were mixed with a "good liquor" in a bottle for 100 days (O'Toole 1997). This product was used similar to soy sauce but would change as the product spread from area-to-area in the Far East. The first written accounts of using soybeans in the recipe was in A.D. 535 and the first record of all the ingredients used in today's soy sauce were published in 1271 and 1368 A.D (O'Toole 1997). The ratio of soybeans to wheat were shown to be around 3:2 to help produce the right ingredients needed for proper flavor (Hui 2012). Production of soy sauce and several other traditional Asian foods are based on the process that is similar to beer production. The use of molds to break down starches and proteins of the grain and other ingredients is similar to the malting of barley and wheat for the use in brewing of beer. Once the sugars and other parts such as amino acids are broken down then the yeast and other microbes can ferment the sugars to the desired product.

In traditional soy sauce production, the soybean and wheat mixture is inoculated with *Aspergillis oryzae* to start the enzyme breakdown (Hui 2012). While that organism is used in production facilities, batches of the product made at home will use wild forms of *Aspergillis* and other wild molds that happen to be on or around the grain or soybean at the start of the enzyme breakdown. Molds and yeast are common on grain products that are uncooked and originate from the field or storage facility. Mold and yeast counts can be found in large quantities on particular grains that have the kernels or berries of the plant open to the air. Certain grains such as sorghum and corn are more open to the air while wheat has a hull that covers the grain.

Once the *koji* or molded soybeans and grain are completely molded and enzymes have had a chance to breakdown the components, *koji* is steeped in a solution of 16-23% salt. The high salt content is needed so no harmful or unwanted bacteria or yeast can grow. The first organisms to grow in the salt solution are lactic acid bacteria, such as *Pediococcus spp.,* that help lower the pH to make the solution more hospitable for yeast to grow and give characteristic flavors (O'Toole 1997). There are different types of yeast that can be found in the making of soy sauce. *Saccharomyces sp.* and *Torulopsis versatilis* are several types of salt tolerant yeast that help use the carbohydrates and amino acids to give the organic acids and flavors that are expected in soy sauce (Hui 2012). Specific kinds of phenolic acids can be brought out during fermentation. In soy sauce production, these acids can give the sauce a smoky, clove-like, woody, or spicy type of flavor. The yeast *Candida versatilis* is the microorganism that causes most of the flavor development in soy sauce (Hui 2012). While these acids bring many wanted flavors, in larger quantities they give off a medicinal phenolic off flavor in soy sauce.

Of soy sauces on the market, one can find a wide variety that cover traditional to gluten-free and low sodium. Kikkoman® is a large worldwide provider of soy sauce and other Asian cuisine condiments. These are traditional soy sauce, sushi and sashimi soy sauce, organic soy sauce, and less sodium soy sauce. All of these kinds have wheat as an ingredient and would have gluten in the solution. As for gluten-free soy sauces, the main one that is called tamari soy sauce. Traditional tamari soy sauce is made with wheat, but several varieties such as one made by Kikkoman® would use sugar instead of wheat to make a true gluten-free tamari soy sauce. Other products are gluten-free sweet soy sauce for rice and low sodium gluten-free tamari soy sauce. The Kikkoman® website does say the ingredients that are used in the product. The gluten-free sweet soy sauce for rice uses sugar, rice vinegar, and yeast extract for flavor and wheat replacement. The low sodium version soy sauce uses vinegar as a way to lower pH so that less salt can be used in making the product.

1.5 Sorghum Grain

Most gluten-free food items use rice as a replacement for wheat as it is very abundant around the world. Rice however can have some downsides that can be a health hazard. As mentioned earlier in this section, rice can be a source of arsenic. This is due to the plant uptake of environmental arsenic. Since this problem can be found everywhere, other grains can be used to replace rice in formulations. One of these grains is sorghum. This grain is widely grown around the world mainly in Africa and Asia with some production in Australia. In the United States, most of the sorghum is produced in the states of Kansas, Texas, Nebraska, and Oklahoma. While most sorghum grown is used for livestock feed, there are several varieties that can be used for human consumption.

In agricultural markets, sorghum grain prices are reflective of the corn markets as stated by the National Sorghum Producers 2016. There is no true sorghum market like corn and the use of sorghum as an alternative to corn is the reason for the way the commodity market views the grain. Sorghum grain is now a growing ingredient in the ethanol industry as a good source of starch for fermentation. The grain can have up to 70% starch in the kernel as compared to 67% found in corn (Ai et al. 2011). Starch would have to be hydrolyzed to be used by yeast or other fermenting microorganisms to make foods such as beer and soy sauce and fuels like ethanol. Since wheat is used in soy sauce production as a main starch source for fermentation, the use of sorghum as a replacement should be adequate as both grains have about similar levels of proteins, around 10.4% for sorghum and 11.6% for wheat, as well as similar starch percentages 70.7% in sorghum and up to 71% in wheat depending on variety (FAO.com 1995).

Sorghum grain can come in several different colors, these include white, red, and purple/black. The problem with the use of sorghum as a foodstuff is the tannins that are contained in the grain. These tannins are used as a mechanism to ward off birds and other animals from eating the grain. Tannins will give a bitter taste to most animals and humans. Most sorghum that is used for food consumption is a white color and does not contain a large amount of tannins (22 μmol/g) compared to the red (140 μmol/g) or purple/black varieties (219 μmol/g) as found in Dykes and Rooney 2006. Some studies done that show that *Lactobacillus* spp. fermentation can breakdown tannins in sorghum into phenolic acid and flavonoids (Svensson 2010). This breakdown will increase the nutritive content of the sorghum. While this study looked at sorghum flour in sourdough bread, soy sauce uses *Lactobacillus* spp. to help with the fermentation and the development of flavors needed for the end product. This shows that several kinds of sorghum flours could be used for the production of soy sauce if the tannins have no impact on the product since they may be are broken down during fermentation.

The other product with hidden gluten is beer. There are several kinds of gluten-free beers that can be found around the world. In Veith 2009, Africa is a leading area that produces gluten-free beer. Since barley and other adjunct for beer is difficult to obtain in certain African countries, sorghum had been used to produce beer. The best sorghum to use for beer brewing is older grain that has been properly stored for several years (Veith 2009). Even with the composition of sorghum and barley are similar, there are a few differences that make malting an entirely different process. As stated in Veith 2009, sorghum starch is similar to corn starch and that during the malting process, starch gelatinization is higher and has less diastatic power than barley malt. With no hull like what is found around barley, sorghum will have different steps in the malting process to account for the lack of hull.

Sorghum is a good substitution for gluten-free bread and other foods. The main protein in sorghum is kafirin. Work done by Smith 2012, the kafarin protein can be used as a substitute for gluten in a visco-elastic resin. Proteins such as zein in corn and kafirin in sorghum can be used as a gluten substitute in dough for breads and other baked products. Under certain conditions kafirin becomes elastic when mixed in warm water which is needed to have a functional dough that compares to conventional wheat flour doughs. This research can be used to help develop better substitutes to replace gluten in a product that gluten has important features such as elasticity and dough adhesion.

As a flour substitute, the whole grain sorghum flour can show different effects on bread characteristics. When sorghum flour is made using different extraction rates (60%, 80% 100%) the extraction percentages being used are the amount of the grain kernel that ends up as flour (Frederick 2007). Whole grain flours are 100% extraction which means you get every part of the grain kernel as flour. Higher extraction flours have a higher fiber and higher starch damage than lower extraction flours. This study was to see how different extraction rates and different milling methods can have an effect of different characteristics in breads. Pin milling is a method that has higher extraction but is not a good source to fraction the flour. Roller milling uses several different types of rolls to reduce to flour to a certain particle size, however, the extraction rate is worse due to larger particles being removed between rolls. The treatment of 60% extraction with pin milling and roller milling showed significant differences in specific volumes (2.54mL/g to 2.40 mL/g), and lower crumb firmness (553.28g to 771.01g) with p-value <0.05 (Frederick 2007). The study was to show how particle size and extraction rate effect the different characteristics of gluten-free bread. As with most flours, the more fiber in the flour the denser and less volume a loaf will have.

To improve the quality of gluten-free foods, sorghum flour can be treated to increase functionality. Heat-treated and ozone-treated flours were tested for loaf characteristics such as volume, color, crumb properties, and crumb firmness, while cake quality for each treatment were specific gravity, volume, symmetry, uniformity, color, crumb structure, and crumb firmness (Marston et al. 2015). Results showed that the ozone-treated flours did not show increased volumes (2.81mL/g to 2.85 mL/g) when the time of ozonation was increased from 15 minutes to 45 minutes (Marston 2006). Heat treatments showed that high heat (125 degrees Celsius) for 30 minutes showed increase of volume in loaf size (3.08 mL/g to 2.62 mL/g) and cells per slice (48.95 cells/cm^2 to 44.87 cells/cm$^{2)}$ and increase in cake volume (72.17 mL/g to 58.5 mL/g) and cells per slice area (79.18 cells/cm^2 to 69.96 cells/cm^2) (Marston et al. 2015). These treatments could be used in sorghum flours to increase the quality of gluten-free baked products.

Products that have volume such as bread or cakes need gluten or other structure forming ingredients to help give volume as well as hold the loaf together. There are some products that do not need volume to value the quality of the product. Tortillas only use flour to help hold things together with little cell structure or volume needed. The use of sorghum flour in the making of tortillas show that tortillas need more extensibility and stretchability to appeal to consumers. Sorghum hybrids and a commercial sorghum flour (Twin Valley Mills sorghum flour) where used in a study; tortillas were evaluated for final weight, diameter, thickness, color, pH, water activity, and moisture content as well as extensibility and strechability (Fernholz 2006). The commercial flour showed better characteristics by a sensory panel of seven trained panelists such as bitterness 3.17 to 4.00 for the other treatments and springiness 3.5 to 2.00. This could have been due to the finer particle size and higher starch damage that had increased water absorption in the commercial flour (Fernholz 2006).

While using sorghum in gluten-free breads and cakes can make an acceptable product, sometimes other ingredients need to be added to increase the quality and other characteristics in the loaf and structure. The use of eggs in a formulation up to 30% can increase consumer's acceptability score up to a 7 and increase volume (Bianchi 2010). Gluten-free breads baked with egg in them showed a higher specific volumes from the control, increase from 2.84 cm^3/g (control) to 2.95 cm^3/g, while the use of antistaling agents (DATEM) showed negative effects (decrease of 0.73 cm^3/g) on volume (Bize et al. 2016). Specific volume shows how much volume is taken up by a certain weight amount such as a gram. Eggs also improved cell structure and produced significant darker crusts ($p<0.05$) and improved overall quality of the product in a consumer panel using a 1 to 9 hedonic scale (Bize et al. 2016).

1.6 Soy Sauce Flavor Profile

To make a product that is similar to what can be found in retail, people that look to make substitutions and omissions to a product worry about the flavor and color of the food they are producing. Certain products can be made from home or made to rival large scale products and can be found in local small markets. With soy sauce the big issue is that there are many different kinds and styles that can be found. Styles such as Thai and Japanese versus what companies made for consumers in the Unites States can be different. In the study done by Cherdchu et al. (2013) sensory panels from different countries were used to describe what flavors are found in many different types of soy sauces found in retail stores in the United States, mainly in the Manhattan, Kansas and Corvallis, Oregon (Cherdchu et al. 2013). This study took 116 different soy sauce products and used them to make a lexicon of flavors that can be found in soy sauce worldwide. Panelists from Kansas State University and Thailand where used to compile the list of flavors for the lexicon for soy sauce (Cherdchu et al. 2013). This study showed that there are 58 different terms that can be used for the description of the flavor in soy sauce. The 58 terms were then ranked into four aspects: common terms, uncommon terms, uncommon characteristics, and complex characteristics. Common terms were used by both panels and had similar definitions such as alcohol, beany, bitter, chocolate, etc. Uncommon terms were terms used by the United States panel that are not found in the Thai language or there was no word to use for them such as brown, caramel, cured, and brown sweet. The one uncommon characteristic of roach was used by the Thai panel but was not used by the United States panel, this can be due to how people live in each country. And the complex characteristics were based on cultural barriers and made it difficult to define what was needed. Terms like dusty, moldy, and earthy were difficult to classify and to differentiate (Cherdchu 2013).

Chapter 2 - Materials and Methods

2.1 Experimental Design

This was a preliminary study to explore the feasibility of using sorghum flour rather than wheat in soy sauce manufacture. Therefore, only one replication was performed and the data was not analyzed statistically for differences.

2.2 Formulation

The formulation used was a basic homemade soy sauce recipe that was found on many how-to sites, as well as several soy sauce articles http://www.wikihow.com/Make-Soy-Sauce 2016). (Table 1) All ingredients were constant and included at the same level except for the types of flour used.

Table 1. Formulations of Control (Wheat) and Sorghum Based Soy Sauces

Ingredients	Control-Wheat Flour	White Sorghum Flour	Waxy Sorghum Flour	Black Sorghum Flour
Cooked Soy Beans	454g	454g	454g	454g
All Purpose Wheat Flour	340g	--	--	--
Nu Life Market White Sorghum Flour	--	340g	--	--
Nu Life Market Waxy Sorghum Flour	--	--	340g	--
Nu Life Market Black Sorghum Flour	--	--	--	340g
Salt	227g	227g	227g	227g
Water	3785mL	3785mL	3785 mL	3785 mL

2.3 Treatments

The one variable that was evaluated in this experiment was the flour used in each treatment. Each treatment used a different kind of flour. These flours included all-purpose wheat flour (Great Value, Walmart, Bentonville Arkansas), white whole grain sorghum flour, white waxy sorghum flour and black whole grain sorghum flour (Nu Life Market LLC, Scott City Kansas). Table 2 shows the proximate analysis of each flour. Each treatment followed the same processing steps and used the same timeframe during molding and fermentation.

Table 2 Proximate Analysis of Flours Used in Treatments

	All-Purpose Wheat Flour	White Whole Grain Sorghum Flour	Waxy Whole Grain Sorghum Flour	Black Grain Sorghum Flour
Total Fat	0%	3.75%	3.75%	3.75%
Total Carbohydrates	80%	77.5%	77.5%	70%
Dietary Fiber	3.33%	5%	5%	10%
Protein	10%	7.5%	7.5%	15%

2.4 Treatment Preparation

The recipe for making the soy sauce was basic with only four ingredients. The first step was to cook the soybeans. Dry food grade soybeans variety WILLCROSS WXE 3386N (Kansas State Agronomy Farm, Manhattan, Kansas) were soaked overnight in water and then brought to boil at 100°C then simmered for 2 hours on low heat the next day. The hulls of the soybeans came off during the boiling process and were scooped off the top of the water with a spoon. After attaining a crumbly and soft texture the soybeans were removed from heat, left cool to room temperature for approximately 2 hours, and then drained of water. A 454g sample was put in a Kitchen Aid Mixer (Benton Harbor, Michigan) and blended for 60 s at low speed until chunky and crumbly. The soybean mash was placed in a mixing bowl Kitchen Aid (Benton Harbor, Michigan), 340g of the particular flour added and mixed for 2 minutes at low speed until a dough was formed.

Figure 1. Mixture of Mashed Soybeans and Wheat Flour

The dough was formed into a log of approximately 13 cm in diameter. Using a sharp knife, about 1.3 cm slices were cut from the log and placed on moistened paper towels laid on baking sheets (Fig. 2). The moisten paper towels were paper towels that were soaked in tap water and squeezed until no water would come out. This paper towel was then used in the molding step. Five total layers of slices separated by moist paper towels were stacked and the whole baking sheet wrapped in Saran Wrap (SC Johnson, Racine, Wisconsin). All treatments were placed in the same warm area about 25°C in 203 Call Hall and left to mold for 14 days.

Figure 2. Slices of Wheat Flour Treatment on Baking Sheet

2.5 Molding and Fermentation

Slices were checked every few days to ensure they were covered in molds at the end of 14 days (Fig. 3) after which they were removed from the paper towels and spread as a single layer with space between slices on a cooling rack for a 3 days till they were dry and hard (Fig. 4).

Figure 3. Wheat flour slice at the end of molding process

Figure 4. Wheat flour slice after drying phase

The dry slices were placed into a salt water solution (Fig. 5). The salt solution consisted of 227g of salt (Morton Kosher Salt) in 3785 mL of water, a 6% concentration. The slices in solution were put in a 1.5 gal Rubbermaid (Atlanta, GA) container covered with a cheesecloth to allow contact with air so that the wild yeast and bacteria to start the fermentation process. For this research, the fermentation was allowed to proceed for a 100 days. Solutions were stirred every 3 days with a spatula or long spoon for 30 seconds so that suspended gases could escape, homogenize the mash, and allow more fermentation.

At the end of the 100 days, liquid soy sauce was decanted and filtered with cheesecloth followed by passing through a French Press (Target, Minneapolis). The solid material, known as koji, was discarded. The sauce collected from each treatment was pasteurized on a stove top to 71˚C and held for 15 seconds.

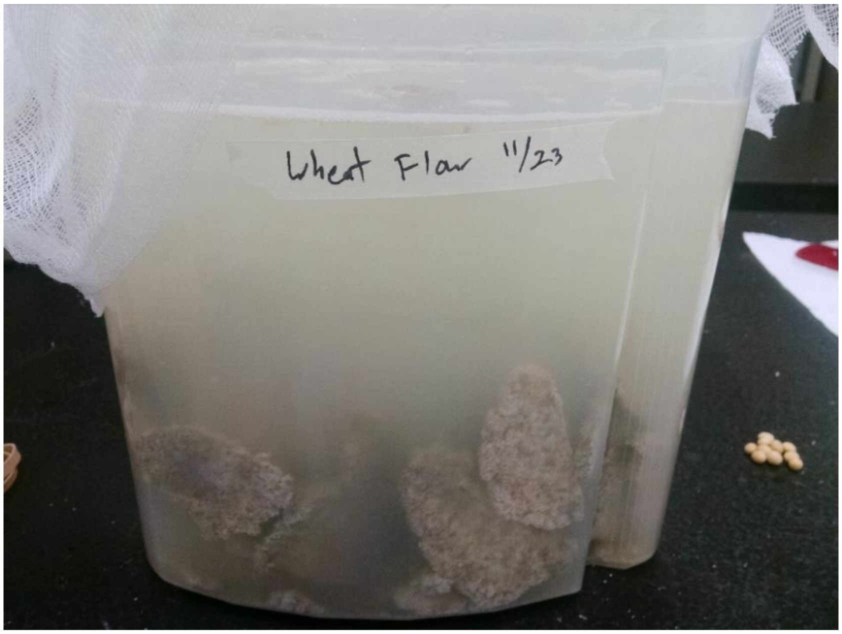

Figure 5. Dried Slices of Wheat Flour in Water Solution

2.6 Testing

Throughout the fermentation process, 15 ml of the supernatant liquid was taken on days 10, 20, 30, 40, 50, 60, 70, 80 90, and 100 and the following tests performed.

2.6a pH

The pH of the liquid was needed to determine how the fermentation was performing or if anything was going wrong. The pH level was measured using an Accumet Portable pH meter (Fisher Scientific, Ashville, NC) with Automatic Temperature Compensation (ATC) electrode. The sauce was placed into a 15mL beaker and pH was read using an electrode that had been calibrated against known buffer solutions of pH of 4 and pH of 7 (AACC Method 02-52.01).

2.6b Salinity

Salinity also was important to see how the proper fermentation was going as stated in the literature review, a higher salt content can inhibit any unwanted microbial growth that can harm. Liquid was taken from the sample and diluted 1:100 dilution due to the high salt content of the sauce and due to the testing equipment can only handle a certain ppm (2000ppm) of salt when testing. The solution was filtered through a number 2 filter paper (Fisher Scientific, Ashville, NC) and then measured by Laqua Pocket Sodium Tester (Horiba Sodium Laqua Pocket Tester, Horiba Kyoto. Japan).

2.6c Color

The color of each liquid was taken on days 50, 60, 70, 80, 90, and 100 to see if there was any change once the fermentation has slowed or has plateaued in terms of pH level. Color was taken using the HunterLab MiniScan (Model Mini Scan EZ 4500L, Hunter Associates Laboratory Inc. Reston, VA) calibrated with white tile and black standard that came with the colorimeter. Values were determined by putting 2 mL of liquid in a small sample dish with a white background. Readings were ran in triplicate to get an average of L*, a*, b* values.

2.6d Sensory Characterization

As mentioned earlier, there is a published flavor lexicon for soy sauce, however, due to monetary and time limits, a trained panel was not used to evaluate the aromas, and the evaluation was performed only by the researcher since this was an exploratory project.

To assess the acceptance and quality of the soy sauces, a consumer study would have been done. The soy sauce would have to be tasted by a group of people to determine if the parameters have been met to say that acceptable soy sauce can be made from gluten-free flours instead of wheat flour However, due to off flavors and aromas developing on all treatments as judged by five students and faculty in Food Science, a decision was made to not allow any product to undergo sensory consumer evaluation.

Chapter 3 - Results, Discussion, and Conclusion

3.1 Results

3.1a pH Level

The pH values for all of the treatments decreased with time which is typical of what should happen in fermentation of soy sauce (Fig. 6). Numbers from these curves show a healthy fermentation that was completed in the first 10-30 days depending on the treatment. All treatments started at a pH value of 7.2, and ended at a pH from 4.18 with the white sorghum flour treatment to a pH of 3.47 with the wheat flour treatment. As found in Hyu (2012), pH of various Japanese soy sauces have pH values from 4.6-4.8. The pH values of the treatments show that there were microorganisms that had the ability to lower the pH in the fermentation lower than commercial soy sauce production. O'Toole (1997) stated that there are optimal pH ranges that are needed for enzymes and certain organisms. Most yeast and bacteria needed in fermentation have a pH range of 3 to 7 with the optimum pH around 4.5 to 6.

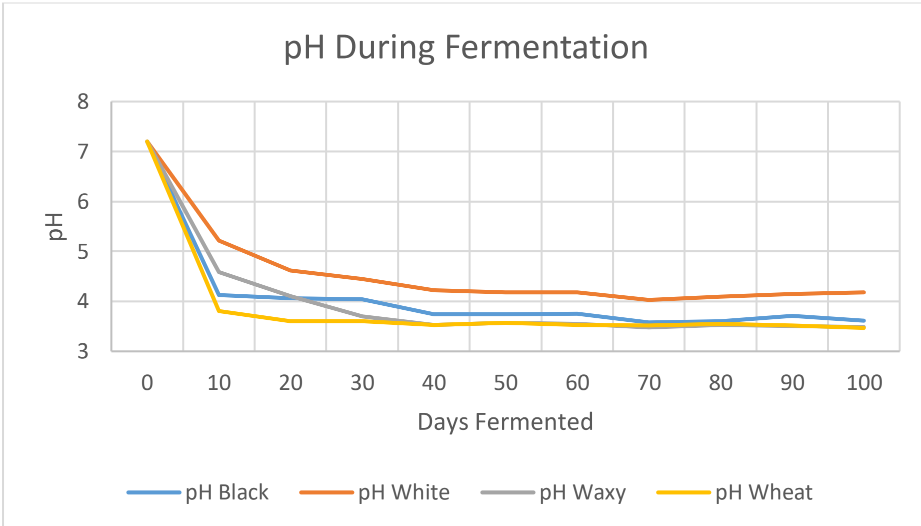

Figure 6. pH of Soy Sauce Made with Black Sorghum, White Sorghum, Waxy Sorghum, or Wheat

The treatment pH's are shown to be lower than the different studies have shown, that could be due to different yeast and bacteria fermenting the soy sauce that can cause off flavors and aromas. As shown in Figure 6. The pH of all treatments had a noticeable drop in the first 10 days of fermentation. Lactic acid bacteria start at the higher pH and are needed to drop the pH to a lower pH so yeast can control the fermentation. The lactic acid bacteria will start at a pH of 6.6 to 7 and drop the solution to below a pH of 5.0 (O'Toole 1997). With the solution at 6% salt, that concentration could be low enough to have other fermenting bacteria and yeast take over that are not wanted in the fermentation or final product. Over time the salt concentration increases which can control certain microorganisms that could ferment the soy sauce. No micro typing was done to determine what bacteria and yeast were present during fermentation. The pH of the white sorghum flour was not as close to the same level (pH 4.18) as the other treatments (pH 3.47-3.62). This difference could be from the lack of enzyme breakdown of the carbohydrates that are available from the beginning molding phase. This could lead one to think that different molds were present that inhibited proper enzyme breakdown.

3.1b Salinity

The salinity of the treatments also were taken every 10 days during the fermentation (Figure 7). While the variation can occur due to the natural evaporation of water over the time of fermentation, the ppm of the samples at the end showed a similar sodium content to regular soy sauce that can be found in the market. As shown in the Material and Methods, the salt content was 6% to begin fermentation. Most soy sauce production has a salt concentration of 10-12% (O'toole 1997). With that lower concentration, as mentioned in pH section, the different types of yeast and bacteria could start fermenting the soy sauce and developing off flavors. Once the solution begins to evaporate off water, then the concentration raises and can then be used to control microbial growth in the fermentation solution. The water started to naturally evaporate off during the fermentation process.

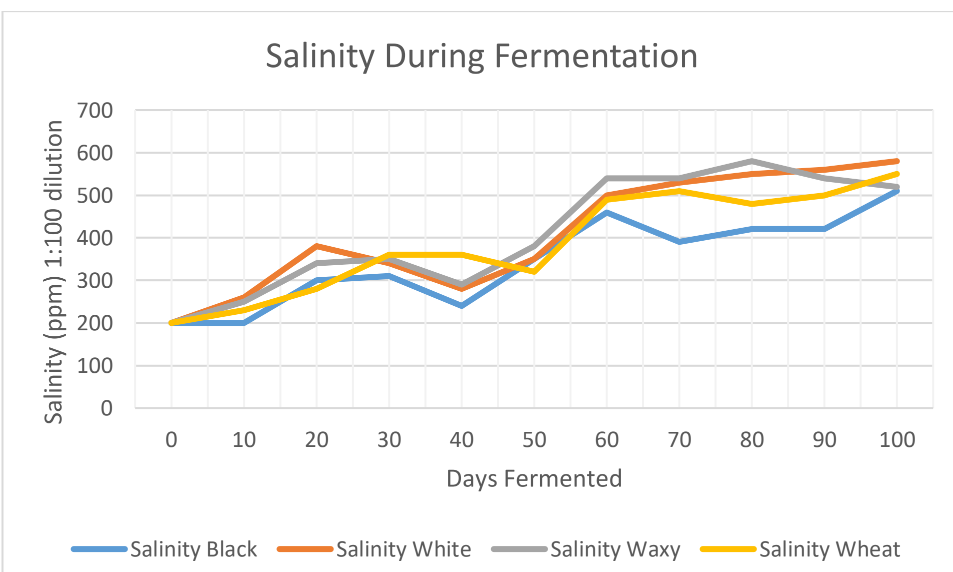

Figure 7. Salinity of Soy Sauce Made with Black Sorghum, White Sorghum, Waxy Sorghum, or Wheat

3.1c Color

No statistical analysis was performed due to the lack of replications for this experimental report. All color data that was recorded are posted (Table 3). As stated in Hyu 2007, the color of most soy sauces can have a range from light brown to a dark redish brown. L* numbers are 0-100 with 0 being black and 100 being white. The a* has no numerical range with positive being red and negative being green. The b* range has no numerical range with positive being yellow and negative being blue. A brown color can be determined by a combination of red and yellow colors. This combination in turn will give a brown to reddish brown color. With this data it can show that there was some proper soy sauce characteristics that could have be forming with the sugars and proteins in fermentation.

Table 3. Color Data from Soy Sauce made with Black Sorghum, White Sorghum, Waxy Sorghum, or Wheat

	Color	Day 50	Day 60	Day 70	Day 80	Day 90	Day 100
Black Sorghum Flour	L*	38.92	41.97	39.73	43.83	41.64	32.58
	a*	12.83	14.28	10.93	12.79	13.25	11.03
	b*	22.70	31.14	22.16	33.11	31.17	18.45
White Sorghum Flour	L*	47.84	40.82	39.24	42.86	42.88	43.98
	a*	11.64	14.84	15.65	14.25	12.41	9.63
	b*	42.91	39.37	39.76	39.52	33.66	29.41
Waxy Sorghum Flour	L*	43.69	41.75	40.36	43.44	44.49	43.40
	a*	12.62	13.39	12.60	12.56	7.42	7.57
	b*	35.13	34.73	31.24	32.97	24.67	22.32
Wheat Flour	L*	49.49	48.81	46.46	48.14	44.67	50.03
	a*	7.40	8.03	9.24	8.35	9.68	7.19
	b*	37.95	39.50	40.77	38.60	29.25	24.92

3.1d Sensory

Due to no consumer panel being ran due to products not being acceptable for human consumption, there was no numerical data from the end of fermentation that could be used to assess the treatments. The fermentation data is the only results that have any impact on the study. Fermentation data does include pH, salinity, color, and changes in aroma as it ferments. The aroma was determined by the researcher as there was not enough time to train other people to help with the study. While no tasting data was taking during fermentation due to health concerns, aroma was carried out so that changes during fermentation can be noted. All aromas followed the lexicon that was stated in the literature review (Cherdchu et al. 2013).

Table 4. Aromas of Soy Sauce made with Black Sorghum, White Sorghum, Waxy Sorghum, or Wheat

Days in Fermentation	Wheat Flour	White Sorghum	Waxy Sorghum	Black Sorghum
0-10	Salty, flour	Salty, Flour	Salty, Flour	Salty, Flour
11-20	Sour beer, lactic	Flour, earthy	Flour, earthy	Sour beer, lactic
21-30	Pickle, vinegar, slight chemical smell	Faint vinegar	Bad, smells rancid, earthy	Floury earthy, lactic
31-40	Bread like, diacetyl	Very sour, vinegary	Sour, vinegar, foot, ferment	Earthy, sour, vinegar
41-50	Heavy diacetyl	Earthy, vinegar	Earthy, vinegar, slight diacetyl	Sweet pickle, vinegar
51-60	Heavy diacetyl	Earthy, bread like, vinegar	Bready like, vinegar	Vinegar, slightly sweet
61-70	Heavy diacetyl	Earthy, Vinegar	Bread like, vinegar	Sweet, vinegar, earthy
71-80	Heavy diacetyl	Earthy, Vinegar	Bread like, vinegar	Sweet, vinegar, earthy

81-90	Heavy diacetyl	Earthy, Vinegar	Vinegar	Sweet, vinegar, earthy
91-100	Diacetyl	Earthy, Vinegar	Vinegar, rancid	Earthy, sour, vinegar

*Observations reported by Researcher only

3.2 Discussion

The sorghum flour used in this study was chosen based off of the variety of sorghum flour that was available from one company. The use of a different color was to see if tannins or anthocyanins can have a play in how the mash ferments and effects the microorganisms that are present. While the soy sauces did not produce suitable product to test with a consumer panel, the fermentation followed the normal fermentation path and showed that if the right conditions and organisms were present then a suitable soy sauce could be produced.

There was an issue that arose during the removal of the liquid from the mash. The removal process was slowed by the fact that the mija was clumping together and making it very hard to get the liquid to go though. Filter paper was not used due to the paper plugging with solid material, so cheesecloth was used to remove the solids as best they could. However, even with using fine mesh screens, there was a small amount of starch or flour still dissolved in the solution. This was not viewed as an issue until the product was heated and several of the treatments began to gelatinize and thicken. Out of all of the treatments, the black sorghum flour did not thicken or gel with flour or starch still in solution. This makes the use of different types of molds and microorganisms in the treatments seem likely as one treatment did not behave like the rest and the final product was different. It also could be explained by the composition of the flour that was used as the black flour possesses a higher fiber content (10% to 3.33% AND 5%) and possesses a higher anthocyanin content (2.0 mg/g) than white, waxy sorghum flour, and wheat flour. As posted on the Nu Life Market LLC. Website, black whole grain flour (http://www.nulifemarket.com/gluten-free/black-whole-grain-sorghum-flour/) has a fiber content of 4 grams per 40 gram sample. White sorghum whole grain flour (http://www.nulifemarket.com/gluten-free/white-whole-grain-sorghum-flour/) and waxy sorghum flour contain 2 grams of fiber per 40 grams. The white all-purpose wheat flour that was as the control contain 1.3 gram fiber per 40 gram serving. The black sorghum flour with the higher fiber content could have had more dissolved fiber particles and most of the starch particles could have been fermented leading to a non-gelatinous liquid that was different from the other treatments.

While there are many reasons why the soy sauce developed the flavors and aromas that made it unfit for human consumption, the main reason for the flavors maybe the use of wild microorganisms that ferment the mash to make a soy sauce. While the use of wild organisms have been used in the making of soy sauce and beer for hundreds of years, not all available bacteria and yeast produce flavors and aromas that are acceptable for humans to consume. A good point to look at is in the beer industry, there are two main flavors that are formed if the beer is "infected" with a bacteria instead of the yeast doing all of the process. The two flavor profiles are diacetyl and vinegar or acetic acid (Charalambous 2013). Both flavors are very pronounced when sampling beer for quality and those bacteria can ruin the beer if they can compete in the ferment with the brewer's yeast (Charalambous 2013). While in the soy sauce there is not have a main organism that is doing the main fermentation, this leaves it open for other salt-tolerant organisms to come in and compete with the wild yeast and *Lactobacillus* to use the available nutrients to make a type of soy sauce. As shown in Table 3, the aromas of the different trials show that there are aroma developments of the two different flavors mentioned above. The confusing part is how only the wheat flour treatment was able to grow more diacetyl while all of the sorghum flour treatments showed a more pronounced vinegar or acetic acid flavor and aroma. All treatments were placed next to each other in the same area so that the wild yeast and bacteria that are needed for fermentation can be as similar for all treatments as possible.

With the split in aromas and flavor it makes one to wonder if there are any yeast and bacteria that are on the different flour during the whole process that can affect the outcome of products such as this. Most flour products are not treated to kill off any microorganisms that can be there as most flour is heated or cooked when making a product. However, this process only had the soybeans being boiled and cooled before the flour was introduced. The molding step in making the soy sauce could lead to bacteria or yeast growing on the slices before the salt brine was used to inhibit certain microbial growth. Each treatment seemed to grow mold slightly different than the others. While the main goal of this step is to breakdown the long chain starches and proteins, the different molds, if they were in fact different, could start a process that could be detrimental to the soy sauce production.

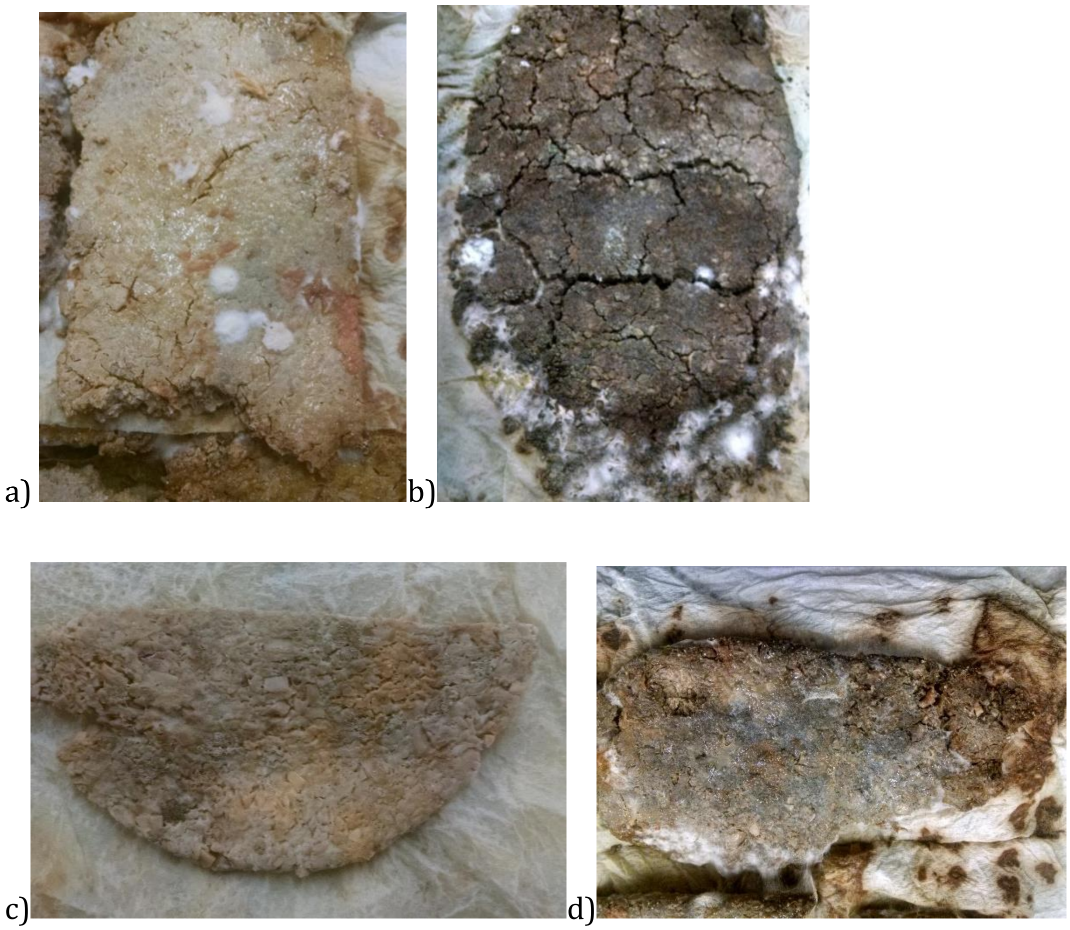

Figure 8. All treatments at 13 days of molding. Top left a) wheat flour. Top right b) waxy sorghum flour. Bottom left c) white sorghum flour. Bottom right d) black sorghum flour

The above figure (Fig. 8) shows the different treatments during the molding process. While there are some similarities, the different coloration of the slices show that there could be a possibility of different molds being present at the time. Only typing of the molds would make it possible to see if the molds are the same or different. While most molds would probably do enzyme secretion needed to make soy sauce, the use of *Aspergillus* sp. could be due to a formation of a flavor compound or that the mold is very good at breakdown of the large starches needed in the process. The *Aspergillus oryzae* used in commercial scale and some small scale productions is used due to its high enzyme secretion and the additional enzymes to help with several organic acid productions (O'toole 1997).

The bacteria and yeast are the main reason for the different flavors and aromas that can arise in the soy sauce. As mentioned earlier in previous chapter, there is a complex set of terms used to describe all the flavors that can be made in soy sauce. While many of the flavors in the lexicon are not very appealing for people when consuming a product, some traditional natural made soy sauces could turn out just like several in the study. This could lead one to think that the use of wild yeast and bacteria can be construed as a guessing game and could limit the potential outcomes. This could be harmful if people are making soy sauce at home and are expecting a product like that found in stores. While the recipe used in this study was pulled off an online site for how to make soy sauce at home, with how these treatment turned out could give some people pause before trying it themselves. While the recipe had some reviews from people saying that the recipe made a respectable homemade soy sauce.

The unknown in microorganisms can make a good product or could in turn make the product taste and smell unappealing or could be harmful to a person's health. The high salt concentrations lower the water activity and make it so only certain bacteria and yeast can grow. Salt solutions up to 18% will produce a solution that has a water activity of around 0.86 (O'toole 1997). While the treatments in the study started out at around 6% salt solution, the eventual evaporation of the water increased the salt concentration to a level high enough to inhibit growth of spoilage or harmful bacteria (O'toole 1997).

However, the use of wild bacteria and yeast still can leave the product in a situation as you cannot control which species are growing in the soy sauce during fermentation. This can make the soy sauce unacceptable and could in turn cause off flavors and compounds that are not wanted in the soy sauce. Some traditional soy sauce is inoculated from the microflora on the walls of the vessels and the facility itself. But, if there was a certain flavor profile you are wanting it can be very difficult to do if you cannot control what species of bacteria and yeast are in the soy sauce.

3.3 Conclusion

Overall the process to make the soy sauce did go as planned. A fermentation of each treatment did follow the normal fermentation path and showed different aromas and slight color changes during the 100 days of fermentation. The use of wild mold, bacteria, and yeast did make it difficult to know if the product was going to turn out. This showed that one should not trust what products can be made with wild microorganisms and should always pasteurize the product before consuming for health reasons. Further research could be done to identify the molds, bacteria, and yeasts that are found in the wild made soy sauce. Also, research could be done to see if finding commercial strains of molds and bacteria can be easily obtained and if that changes how the process goes when made at home or in a lab setting. Caution should be taken when making soy sauce at home as the flavors and aromas can be overpowering and the soy sauce should not be consumed raw.

References

Ai Y, Medic J, Jiang H, Wang D, Jane J. 2011. Starch characterization and ethanol production of sorghum. J Agric Food Chem 59(13):7385-92.

Bianchi MP. 2013. Development and improvement of sorghum-based gluten-free dinner rolls. Manhattan, Kan.: Manhattan, Kan. : Kansas State University.

Bize, M., Smith, B., Aramouni, F. and Bean, S. 2016. "The Effects of Egg and Diacetyl Tartaric Esters of Monoglycerides Addition on Storage Stability, Texture, and Sensory Properties of Gluten-Free Sorghum Bread'. Journal of Food Science. JDFS-2016-1325 R1. 7JFS: Sensory and Food QualityCFR - Code of Federal Regulations Title 21. Accessdata.fda.gov. 2017 [accessed 2017 Apr 17]. https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?fr=104.20

Charalambous G. 2013. Off-flavors in foods and beverages. Elsevier.

Cherdchu P, Chambers E, Suwonsichon T. 2013. Sensory lexicon development using trained panelists in thailand and the U.S.A.: Soy sauce: Lexicon development by two panels for soy sauce. J Sens Stud 28(3):248-55.

Dykes L and Rooney LW. 2006. Sorghum and millet phenols and antioxidants. J Cereal Sci 44(3):236-51.

Fernholz MC. 2008. Evaluation of four sorghum hybrids through the development of sorghum flour tortillas. Manhattan, Kan.: Manhattan, Kan. : Kansas State University.

Frederick EJ. 2009. Effect of sorghum flour composition and particle size on quality of gluten-free bread. Manhattan, Kan.: Manhattan, Kan. : Kansas State University.

Gluten-free White Whole Grain Sorghum Flour. Nu Life Market. [accessed 2017 Mar 3]. http://www.nulifemarket.com/gluten-free/white-whole-grain-sorghum-flour/

Gluten-free Black Whole Grain Sorghum Flour. Nu Life Market. [accessed 2017 Mar 3]. http://www.nulifemarket.com/gluten-free/black-whole-grain-sorghum-flour/

Green PH, Lebwohl B, Greywoode R. 2015. Celiac disease. J Allergy Clin Immunol 135(5):1099-106.

Gums and Stabilisers for the Food,Industry Conference. 2012. Gums and stabilisers for the food industry 16. Cambridge, Eng.: Cambridge, Eng. : RSC Publishing.

Hui YH(. 2012. Handbook of plant-based fermented food and beverage technology. 2nd edition.. ed. Boca Raton, FL: Boca Raton, FL : CRC Press.

Lafiandra D. 2004. Gluten proteins. Cambridge: Cambridge : Royal Society of Chemistry.

Marston, K., Khouryieh, H. and Aramouni, F. 2015. Effect of heat treatment of srghum flour on the functional properties of gluten-free bread and cake. LWT-Food Science and Technology 65 (2016)637-644O'Toole DK. 1997. The role of microorganisms in soy sauce production. Adv Appl Microbiol 45:87-152.

P. Basis Basics. Sorghum Growers. 2012 Sep [accessed 2017 Mar 30]. http://sorghumgrowers.com/basis-basics/

Reilly NR. 2016. The gluten-free diet: Recognizing fact, fiction, and fad. J Pediatr 175:206-10.

Sato K and Ueno S. 2014. Physical properties of fats in food.

Sorghum and millets in human nutrition. Sorghum and millets in human nutrition. [accessed 2017 Mar 30]. http://www.fao.org/docrep/t0818e/T0818E0b.htm

Smith BM. 2012. Functionality of corn and sorghum proteins in visco-elastic dough systems. Manhattan, Kan.: Manhattan, Kan. : Kansas State University.

Svensson L, Sekwati-Monang B, Lutz DL, Schieber A, Gänzle MG. 2010. Phenolic acids and flavonoids in nonfermented and fermented red sorghum (sorghum bicolor (L.) moench). J Agric Food Chem 58(16):9214-20.

Tanner G, Colgrave M, Blundell M, Goswami H, Howitt C. 2013. Measuring hordein (gluten) in beer - A comparison of ELISA and mass spectrometry. PLoS One 8(2).

wikiH. How to Make Soy Sauce. wikiHow. 2016 [accessed 2016 Nov 10]. http://www.wikihow.com/Make-Soy-Sauce

Veith KN. 2009. Evaluation of four sorghum hybrids through the development of gluten-free beer. Manhattan, Kan.: Manhattan, Kan. : Kansas State University.

Appendix A - Commercial Soy Sauce Flow Diagram

Picture 1. Flow Chart of Commerical Soy Sauce Production form Plant-Based Fermented Foods and Beverage Technology. Second Edition. Page 95.

Hui YH(. 2012. Handbook of plant-based fermented food and beverage technology. 2nd edition.. ed. Boca Raton, FL: Boca Raton, FL : CRC Press